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TRIFOLIUM KENTUCKIENSE (FABACEAE, PAPILIONOIDEAE), A NEW SPECIES FROM FRANKLIN AND WOODFORD COUNTIES, KENTUCKY

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ABSTRACT

Trifolium kentuckiense, which is most similar to *Trifolium reflexum,* is described as a new species. It is known from one population in each of Franklin and Woodford counties, Kentucky. The two species differ in growth habit, petiole length, stipule width, sepal lobe length, and peduncle-terminal petiole length ratio. *Trifolium kentuckiense* is named for the Commonwealth of Kentucky, to which it is apparently endemic.

KEY WORDS: *Trifolium kentuckiense*, *Trifolium reflexum*, new species, Kentucky

Species of the genus *Trifolium* are annual, biennial, or perennial herbs with alternate, palmately (rarely pinnately) compound leaves, most commonly with 3 leaflets. The genus encompasses approximately 240 species (Zohary & Heller 1984), though recent studies in New World clovers indicate that there may actually be many more species. Clovers are found in warm to cold temperate environments in both hemispheres, with centers of endemism in Mediterranean habitats (Isley 1990; Zohary & Heller 1984). In the flora of North America north of Mexico, there are 64 native and at least 29 introduced species of *Trifolium* (Isely 1998). Eleven species of the genus have been previously documented from Kentucky (Vincent 2001), only 2 of which are native to that state.

Trifolium reflexum (Buffalo clover) is an annual to biennial herb 20-50 cm tall, with reflexed flowers in inflorescences on long peduncles with corollas ranging from deep pink to white (Vincent 2001). The species has an historical range throughout eastern North America, including the states of Florida, Iowa, Kansas, Kentucky, Maryland, Nebraska, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Texas, Virginia, West Virginia (Vincent 1991, 2001; Warren et al. 1986). Buffalo clover is becoming rare in much of its range, and is now listed as rare or endangered in Illinois, Indiana, Kentucky, Maryland, Ohio, Pennsylvania, and Tennessee (Vincent 2001; Kentucky State Nature Preserves Commission 1996; USDA NRCS 2013).

In the course of field work in Kentucky, two populations of a clover similar to *Trifolium reflexum* were found independently by Tara R. Littlefield (Franklin County) and Joe Lacefield (Woodford County). Collections were made subsequently by Daniel Boone and Julian Campbell. This new material was sufficiently different from other *T. reflexum* specimens that it seemed potentially a previously undescribed species.

Materials and Methods

Measurements were made for 20 characters from 4 herbarium specimens of the potential new taxon and from 38 specimens of *T. reflexum* from throughout the range of that species. Flowers were rehydrated in a 1% dilution of Aerosol OT in distilled water before dissection. Measurements were made using an ocular micrometer in a compound microscope and with a metric ruler. Statistical analyses (mean, standard deviation, and TTEST) were conducted using Microsoft Excel 2010.

Principal Components Analysis (PCA) and sequential, agglomerative, hierarchical, non-overlapping (SAHN) clustering analysis were conducted using NTSYS-pc (Rohlf 2000).

Results

Statistical analyses revealed significant differences ($\alpha = 0.05$) in the following characters: stipule width, terminal petiole length, petiole length, and sepal lobe length (Table 1). Stem habit and peduncle-terminal petiole ratio were also included in Table 1 because of their importance as field characters. *Trifolium kentuckiense* has decumbent stems and terminal petioles that are about four times as long as the peduncles, on average, while *Trifolium reflexum* has erect or ascending stems and petioles that are about a third as long as the peduncles, on average (Table 1). The ranges of terminal petiole length and the ratios of peduncle length to terminal petiole length do not overlap. Stipule width, petiole length, and sepal lobe length have ranges that overlap, but their means, medians, and standard deviations do not. A PCA plot (Figure 1) and a dendrogram resulting from cluster analysis (Figure 2), based on analyses of 20 characters, show clear delineation between *T. reflexum* and *T. kentuckiense*. There were no intermediate specimens observed in this study that would indicate any hybrids or gene flow between the two species.

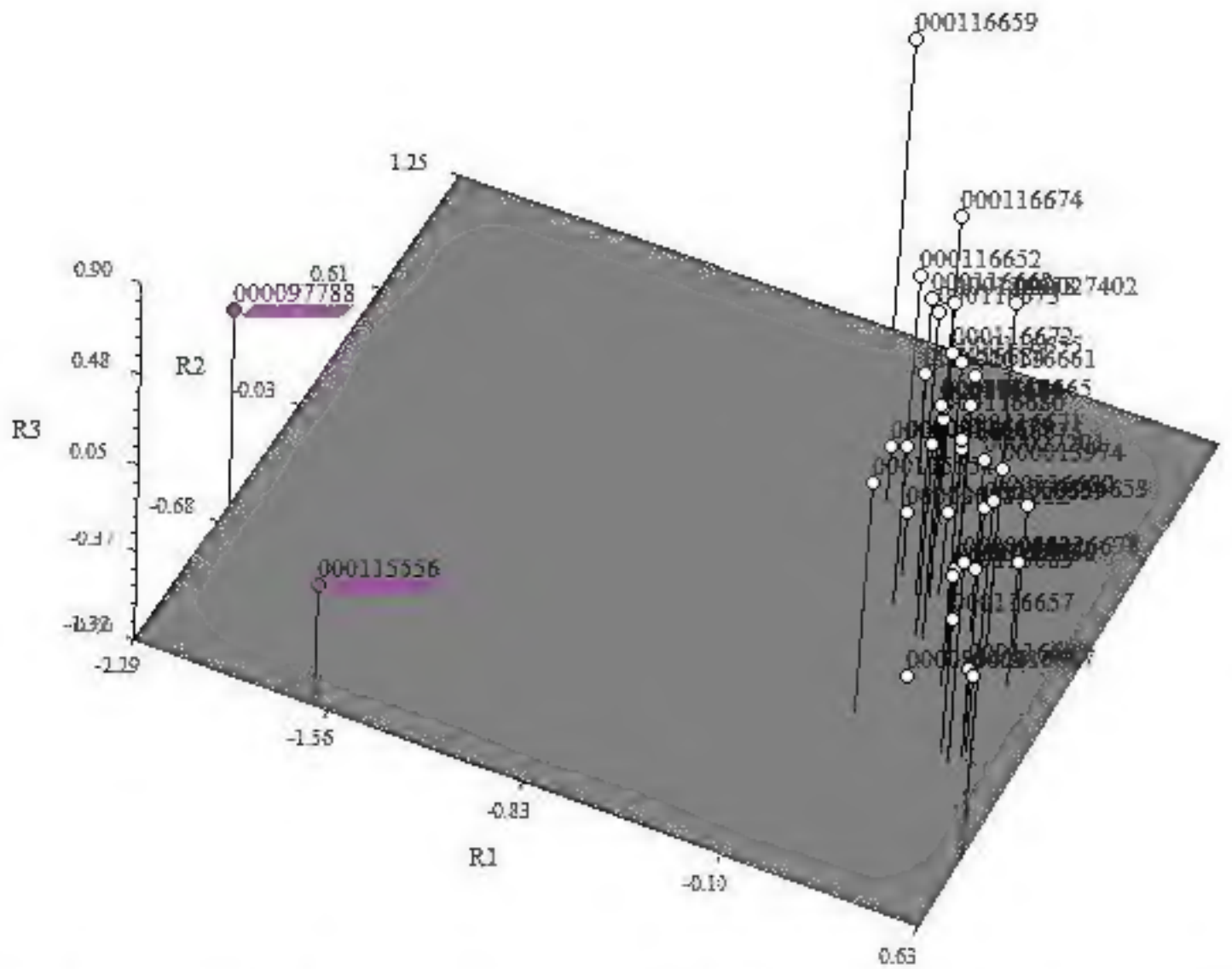


Figure 1. Three dimensional plot derived from PCA, showing *Trifolium kentuckiense* (underlined in purple) separated from *Trifolium reflexum* (no underline). Each number refers to a barcode on a specimen.

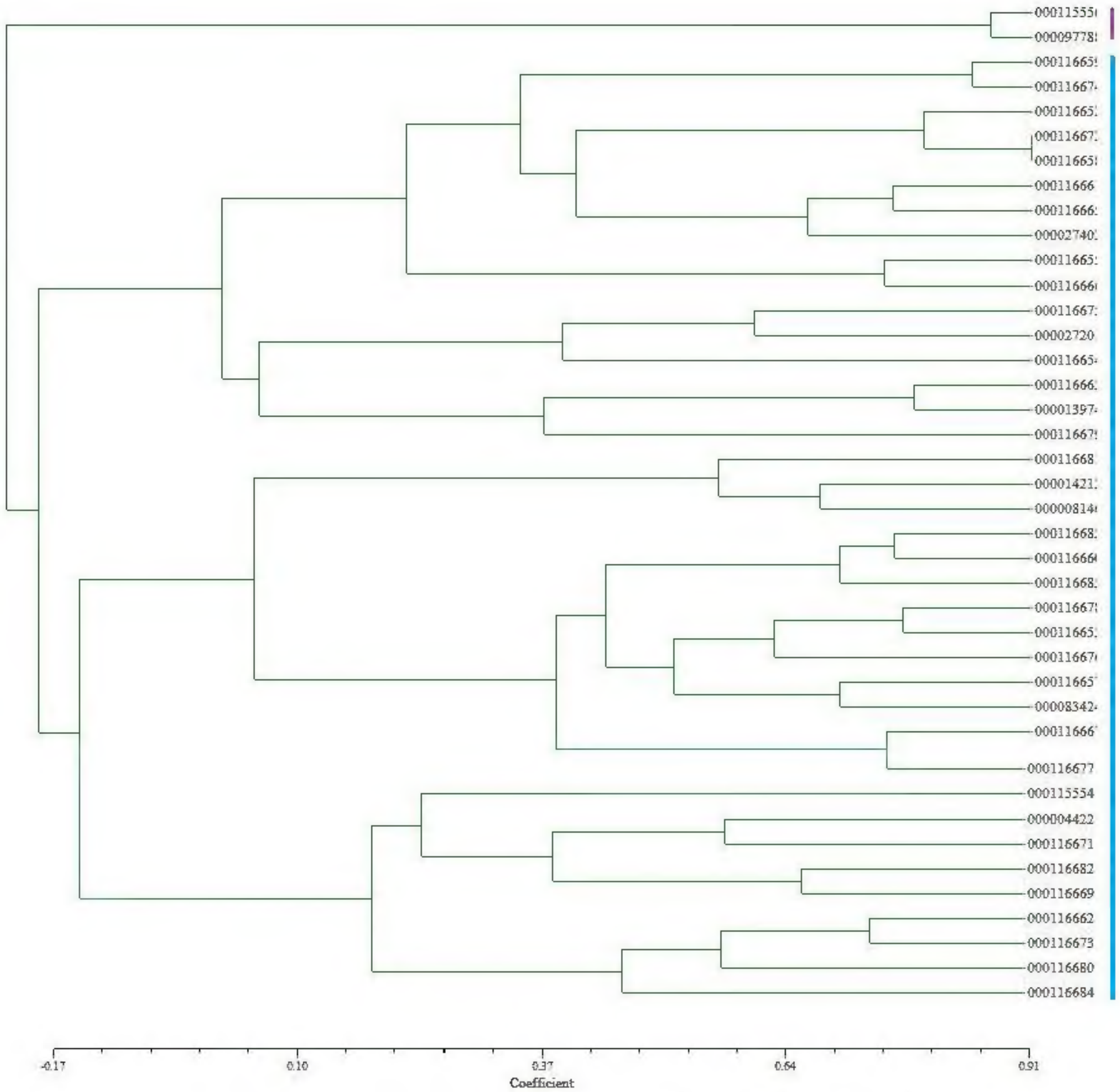


Figure 2. Dendrogram resulting from SAHN cluster analysis showing *Trifolium kentuckiense* specimens (purple) clustering separately from *T. reflexum* specimens (blue). Each number refers to a barcode on a specimen.

Table 1. Differentiating characters of *Trifolium kentuckiense* and *Trifolium reflexum*. The growth habit is described. The range and mean values for stipule width in cm (StipW), terminal petiole length in cm (TermPetL), petiole length in cm (PetL), sepal lobe length in mm (SepLopL), and peduncle-terminal petiole length ratio (PedL/PetL) are listed; *statistically significant ($\alpha = 0.05$).

	T. kentuckiense	*T. reflexum*
Stem Habit*	decumbent	erect/ascending
StipW*	(0.5–) 1.1(–1.4)	(0.3–) 0.7(–1.3)
TermPetL*	(5.9–)7.3(–8.2)	(0.3–) 1.7 (–3.5)
PetL*	(3.5–)10.7(–18)	(0.7–) 3.9(–8.5)
SepLobL*	(0.2–) 0.29 (–0.37)	(0.3–) 0.45 (–0.7)
PedL/PetL	(0.23–) 0.26(–0.31)	(0.4–) 1.31(–4.2)

Trifolium kentuckiense Chapel & Vincent, **sp. nov.** Figures 3, 4. **TYPE: USA. Kentucky.** Woodford Co.: In open limestone woods close to Clear Creek alongside tributary, between Rt. 33 and Keene-Troy Pike, NW of Ebenezer Cemetery, N of Troy, 18 May 2010, *D. Boone & J. Campbell s.n.* (holotype: MU 270867; isotype KNK).

Similar to *Trifolium reflexum* L. in its biennial life cycle, large leaflets, large inflorescences, and flowers that are 1–1.5 cm long, but differing from it in the following characters: decumbent habit, terminal petiole lengths ranging from 5.7–8.2 cm (as opposed to 0.3–3.5 cm), sepal lobe lengths ranging from 0.2–0.37 cm (as opposed to 0.3–0.7 cm), and peduncle-terminal petiole length ratios ranging from 0.23–0.31 (as opposed to 0.4–4.2).

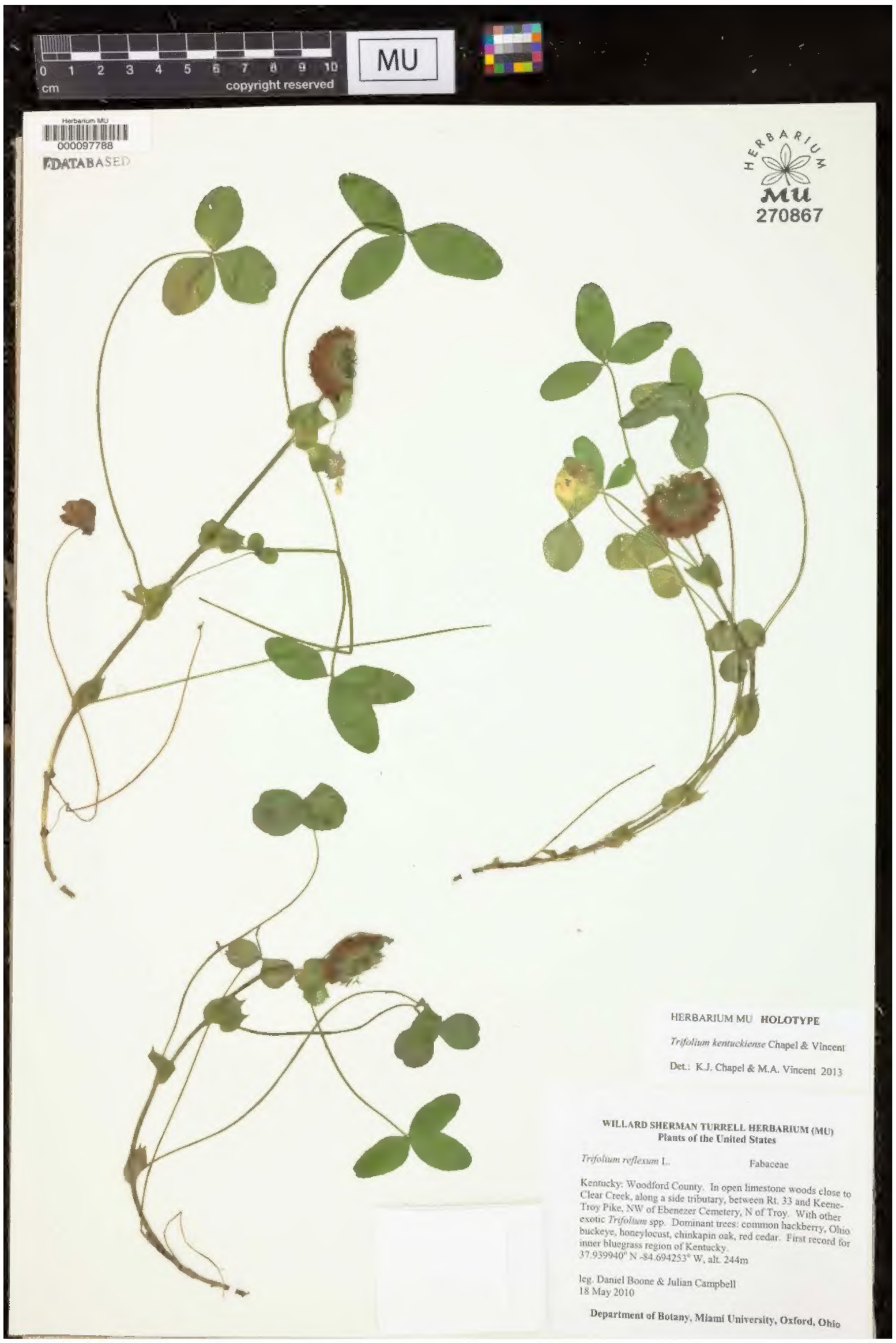
Plants annual; stems branched from the base, decumbent, 15–29 cm long, sparsely to moderately pubescent, arising from fibrous tap roots. **Stipules** thin, foliaceous, persistent, 1–1.6 X 0.5–1.4 cm, broad at base, coming to an abrupt point at the apex, or more gradually acuminate, adnate to petioles for half their length, each with a prominent auricle above the adnate portion. **Leaves** trifoliate; petioles 3.5–16 cm, sparsely pubescent; leaflets shortly petiolulate; petiolules moderately to densely pubescent, 1 mm long; leaflets glabrous, elliptical to obcordate, 0.6–3.5 X 0.6–2 cm, margins dentate to undulate. **Peduncles** short, 2–3 cm long. **Inflorescences** globose, 2–2.8 X 1.2–2.8 cm, 25–40 flowered, flowers on pedicels 0.35–0.5 cm, pedicels soon reflexing. **Calyx tubes** five-lobed, sinuses u-shaped; lobes 2–5 X 0.5–0.9 mm, longer than the tubes. **Corollas** white, 0.85–1.02 cm long; banners obovate, apices rounded to emarginate. **Ovaries** glabrous; ovules 3. **Fruits** 3.75-4.8 mm X 1.65–2.55 mm, 1–3-seeded. **Seeds** 1.35-1.5 mm, globular, smooth, light brown. Flowering April to May. Named in honor of the Commonwealth of Kentucky, in which it was discovered.

Paratypes. **Kentucky**. Fayette Co.: cultivated at 3525 Willowood Road, Lexington, seed from Woodford County site, 14 June 2011, *J. Campbell s.n.* (MU). Franklin Co.: along Ninevah Rd., about 800 ft N of where road leaves Little Benson Creek, 38.105556° N, 84.879722° W, 9 May 2012, *T.R. Littlefield s.n.* (MU).

Trifolium kentuckiense is most morphologically similar to *Trifolium reflexum* and likely very closely related to that species, though their precise relationship is not yet determined. The new species differs from *T. reflexum* in growth habit, stipule width, sepal lobe length, petiole length, and terminal petiole length. The new species has decumbent stems while *T. reflexum* has erect or ascending stems (Vincent 1991). The prominent auricle on stipules of *T. kentuckiense* adds significant width. The sepal lobes of *T. kentuckiense* are shorter than those of *T. reflexum.* The petioles of *T. kentuckiense* are considerably longer. The peduncle-terminal petiole ratio is not statistically significant, but it is an important character for identification. The peduncles of *T. kentuckiense* are much shorter than the petioles. The measurements taken of *T. reflexum* did not overlap with the ones taken from the new material. In addition, the known populations of *T. kentuckiense* occur on limestone based soils, whereas most populations of *T. reflexum* are found on acidic soils.

The two known populations of *Trifolium kentuckiense* (which are about 25 miles apart) were collected in Franklin and Woodford counties, Kentucky, on limestone based soils. The Franklin County population was found growing on a SW-facing steep slope above a small tributary. Associates included *Aesculus glabra, Alliaria petiolata, Astranthium integrifolium, Blephilia ciliata, Brachyelytrum erectrum, Camassia scilloides, Campanulastrum americanum, Carex blanda, Carex oligocarpa, Cercis canadensis, Dasistoma macrophylla, Desmodium cuspidatum, Dodocatheon meadia, Fleischmannia incarnata, Fraxinus quadrangulata, Juniperus virginiana, Lesquerella globosa, Lonicera japonica, Lonicera maackii, Nothoscordum bivalve, Penstemon digitalis, Perideridia americana, Ptelea trifoliata, Polygyala latifolia, Quercus muhlenbergii, Scutellaria ovata, Sedum pulchellum, Silene caroliniana, Tradescantia subaspera, Zanthoxylum americanum.*

The Woodford County population was found growing in open limestone woods, also near a tributary. Other exotic *Trifolium* species were present. Dominant trees included *Aesculus glabra*, *Celtis occidentalis*, *Gleditsia triacanthos*, *Juniperus virginiana*, *Quercus muhlenbergii*. *Trifolium reflexum* has not been found in either of these counties but is found in the adjacent counties of Fayette and Mercer (Vincent 2001).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Trifolium kentuckiense Chapel & Vincent
Det.: K.J. Chapel & M.A. Vincent 2013
WILLARD SHERMAN TURRELL HERBARIUM (MU)
Plants of the United States
Trifolium reflexum L. Fabaceae
Kentucky: Woodford County. In open limestone woods close to Clear Creek, along a side tributary, between Rt. 33 and Keene-Troy Pike, NW of Ebenezer Cemetery, N of Troy. With other exotic Trifolium spp. Dominant trees: common hackberry, Ohio buckeye, honeylocust, chinkapin oak, red cedar. First record for inner bluegrass region of Kentucky.
37.939940° N -84.694253° W, alt. 244m
leg. Daniel Boone & Julian Campbell
18 May 2010
Department of Botany, Miami University, Oxford, Ohio

Figure 3. Holotype of *Trifolium kentuckiense, Boone & Campbell s.n.* (MU).

Figure 4. *Trifolium kentuckiense*, showing habit, from the site in Woodford County, Kentucky. Photo courtesy of Joe Lacefield.

ACKNOWLEDGEMENTS

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